Functional characterization of the origin binding domain of the replication initiator RepB: identification of the residues involved in the specific recognition of the cognate origin and in the catalytic activity

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ABSTRACT:

Several mutants in the origin-binding domain of RepB, the initiator of replication of plasmid pMV158, were constructed and purified. Analysis of these mutants provided information on the structural motifs of RepB involved in the specific recognition of the plasmid origin and on the role of different residues of the catalytic site.

Keywords: Rolling circle replication, replication initiator, protein-DNA binding, endonuclease.

1. Introduction

Initiation of rolling circle replication (RCR) of the streptococcal promiscuous plasmid pMV158 requires cleavage of one DNA strand at a specific site within the double-strand origin (*dso*) by the plasmid-encoded RepB protein. The *dso* consists of the *nic* locus, which includes the inverted repeat IR-I containing the nick sequence in the spacer DNA, and the *bind* locus, which comprises 3 tandem distal direct repeats (DDR). RepB binds with high affinity to the *bind* locus and promotes extrusion of hairpin IR-I, wherein the nick sequence becomes unpaired and hence accessible for nicking [1]. RepB-catalyzed nicking at the *dso* provides a 3'-OH end that is used as a primer for leading-strand synthesis by the bacterial replisome. RepB forms a homohexameric ring [2] where each protomer consists of a C-terminal oligomerization domain (OD), and an N-terminal origin-binding domain (OBD) containing the DNA-binding and endonuclease activities [2]. We have initiated the functional characterization of OBD by identifying the structural elements involved in origin recognition as well as the residues of the active site involved in the RCR initiation and termination steps.

2. Results and Discussion

Characterization of the two separate RepB domains demonstrated that the catalytic and DNA binding activities of the OBD can be uncoupled from the hexamerization potential located in the OD. Superposition of the structure of the OBD with that of the dsDNA-AAV5 Rep complex [3] showed that helix α2 and the N-terminal tail of OBD were properly positioned to contact the dsDNA. To test this hypothesis, we generated various OBD mutants wherein some residues of these structural motifs were replaced by Ala, and studied its ability to *bind* to the *bind* locus (Fig. 1). The results confirmed our initial hypothesis and showed that the dsDNA binding capacity can be altered without affecting the endonuclease and strand-transfer activities of OBD.

Binding of Mn^{2+} to the active site is essential for the catalytic activity of RepB and involves 4 protein ligands and a single-solvent molecule. Substitution of the catalytic Tyr99 by Ala produced a severe decrease in the endonuclease and strand-transfer activities of the protein on ssDNA. To study in more detailed the catalytic mechanism of the protein, we replaced some residues of the active site by Ala and evaluated their contribution to the endonuclease and strand-transfer activities of OBD. The results showed that Tyr115 could participate in catalysis probably by modulating the charge of the metal ligand Asp42. In addition, His102 could promote the nucleophilic attack of Tyr99 on the phosphorus.

The data presented in this work partially overcome the lack of structural information about the RepB-*bind* locus interface involved in the specific recognition of the cognate plasmid origin, and contribute to the elucidation of the catalytic mechanism of the initiation of replication mediated by RepB.

References

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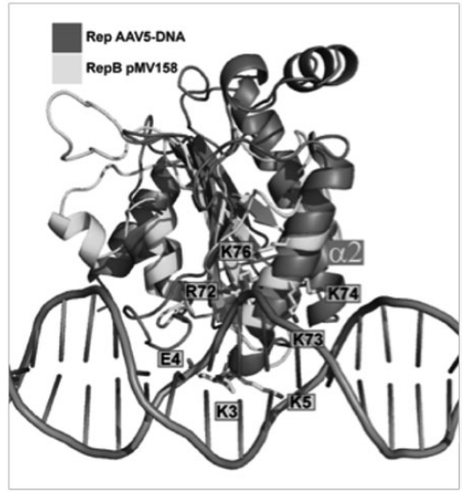

Fig. 1: Superposition of the structure of the OBD of RepB with that of the dsDNA-AAV5 Rep complex. Residues of the helix α2 and the N-terminal tail of OBD that were properly positioned to contact the dsDNA are indicated.